FIGURE 1

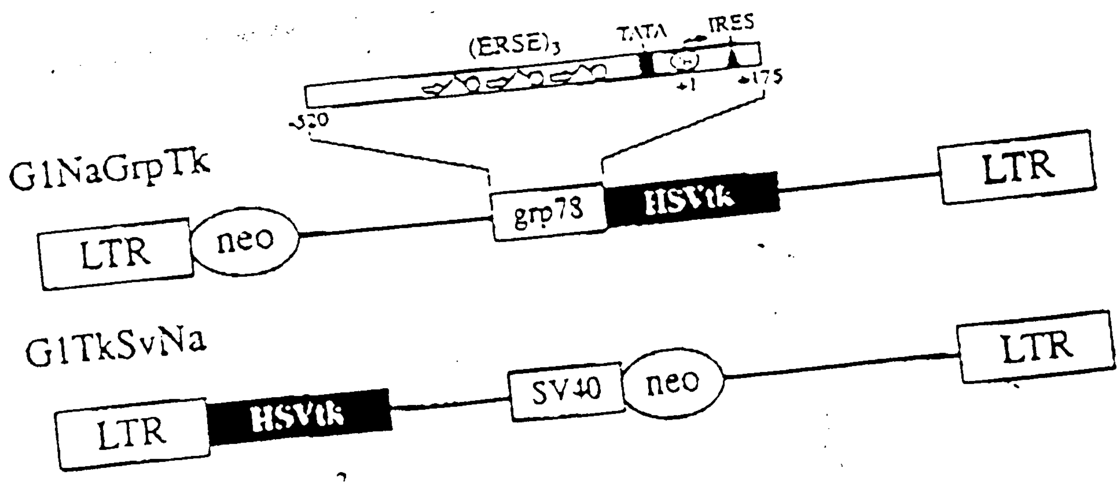

Figure 1 shows a schematic drawing of the recombinant retroviral vectors. In the G1NaGrpTk vector, the MuLV LTR drives the expression of neomycin phosphotransferase (neo) gene that is used as a selection marker. In this same vector, the grp78 promoter, spanning (-520 to +175) drives the HSVtk gene. The grp78 promoter fragment contains three copies of the endoplasmic reticulum stress element (ERSE), the TATA box, and an internal ribosome entry site (IRES) in the 5' untranslated region downstream of its transcription initiation site (+1). In the G1TkSvNa vector, the MuLV LTR drives expression of the HSVtk gene, while the SV40 promoter drives the neo gene.

FIGURE 2

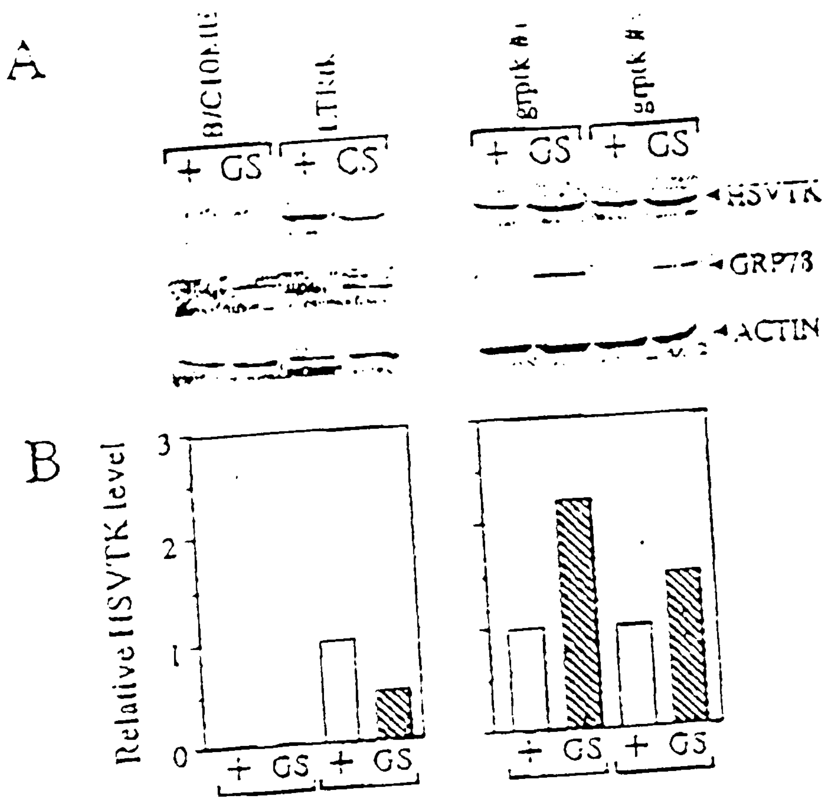

Figure 2 shows induction of HSVtk by the grp78 promoter under glucose starvation conditions. Panel A shows equal amounts of cell lysates from the parental B/C10ME cells, independently derived clonal cell lines transduced with G1TKSvNa (LTRtk#5), or transduced with G1NaGrpTk (grptk#1 and grptk#3) were subjected to Western blot analysis with antibodies against HSVtk, GRP78 and b-actin. The cells were grown under normal culture medium (+) or glucose-starved (GS) conditions for 24 h. Panel B shows a bar graph indicating the intensity of the protein bands quantitated by densitometry and normalized against that of actin serving as an internal loading control. The relative levels of HSVtk under normal culture or glucose-starved conditions were plotted below the autoradiograms, with the protein level in control cells set as 1.

FIGURE 3

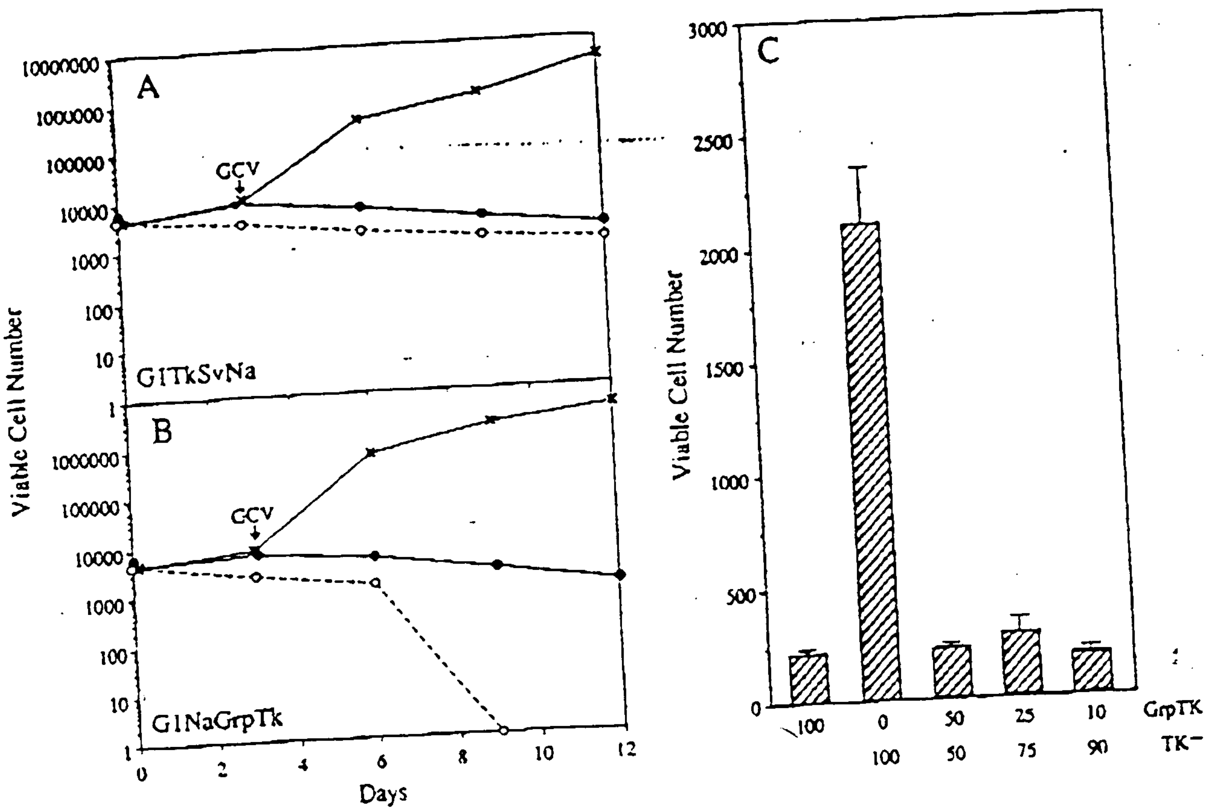

Figure 3 shows the results of an in vitro GCV-sensitivity assay for B/C10ME cells. Panel A is a line graph showing about 5 x 103 of G1TkSvNa/clone #3 clones were seeded in duplicate into 6-well plates and incubated without (X) or with 0.1 (closed circles, open circles) mg/ml GCV starting at day 3 as indicated. The cells were then incubated in normal medium (—) or pretreated in glucose-free medium (- - -), and the number of surviving cells were determined by the trypan blue exclusion method. Panel B shows data generated by the procedure used in A, except that G1NaGrpTk/clone #3 cells were used. Panel C shows *in vitro* bystander effect, non-transduced B/C10ME cells (TK–) were co-cultured with different ratio of B/C10ME clonal cell lines stably transfected with G1NaGrpTk. A total of 3,000 cells with various ratios were plated in quadruplicate in 96 well plate and treated with 10 mg/ml GCV for 10 days. The number of remaining viable cells was measured by cell proliferation assay.

FIGURE 4

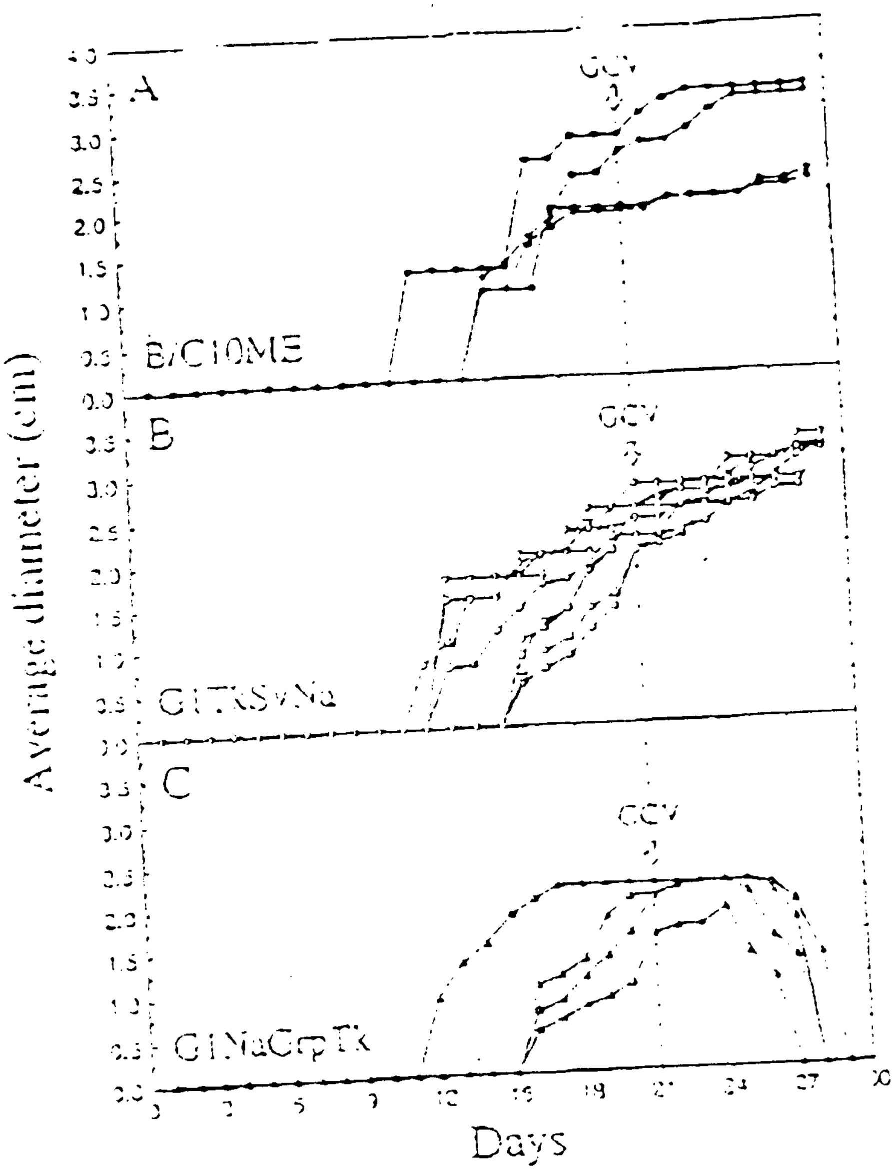

Figure 4 shows tumor growth curves for B/C10ME fibrosarcoma. Panel A shows B/C10ME cells. Panel B shows three independently derived G1TkSvNa clonal derivatives (#2, #3, #5). Panel C shows two independently derived G1NaGrpTk clonal derivatives (#1, #3) were used. Equivalent numbers of 2 x 107 viable cells were subcutaneously injected into BALB/c mice. Bi-perpendicular measurements were taken over a period of 29 days. GCV (as indicated by arrows) was administered daily starting at day 21 at a dosage of 100 mg/kg of body weight.

FIGURE 5

Figure 5 shows immunohistochemistry staining of HSVtk protein expression in B/C10ME tumor tissues from mice. Panel A shows that, after counterstaining the tissue section with methyl green, no DAB stain can be detected in tumor from non-transduced B/C10ME cells. Panel B shows isolated patches of HSVtk protein expression can be observed by cytoplasmic brown DAB staining in tumor from B/C10ME cells transduced with G1TkSvNa. Panel C shows high level of HSVtk protein expression as shown by dark cytoplasmic brown DAB staining in tumor from B/C10ME cells transduced with G1NaGrpTk. The magnification is 200X.

FIGURE 6

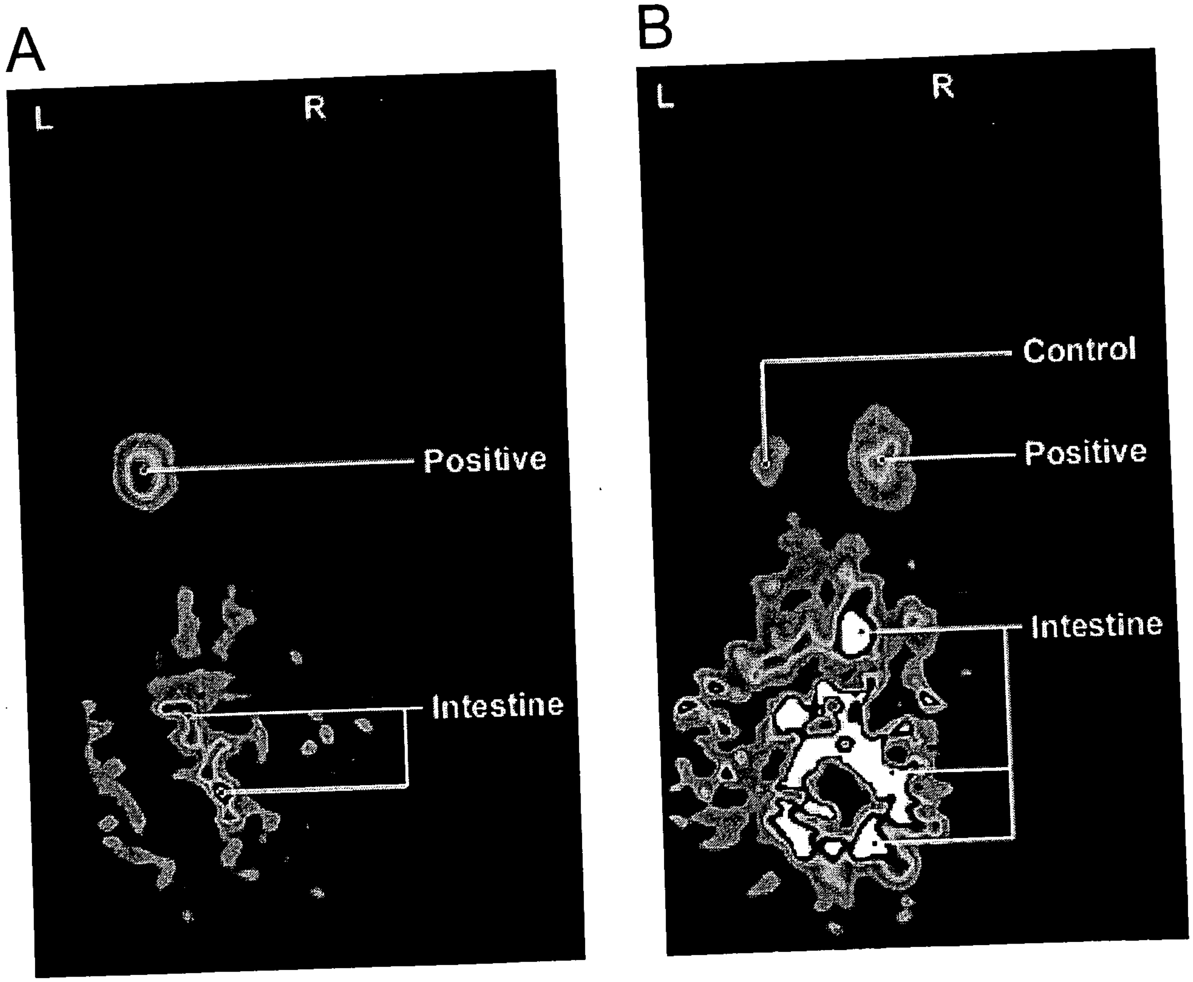

Figure 6 shows micropet images of hypoxia inducible HSVtk expression in a murine mammary adenocarcinoma model. The mice were bearing tumors derived from a murine mammary adenocarcinoma cell line, TSA, which has been stably transfected with a retroviral vector, G1NaGRP-HSVtk, containing the GRP78 promoter that drives HSVtk gene expression. The mcroPET scan was performed as described (Gambhir S. S. *et al.* PNAS, 96:2333-2338, 1999). The isotope-labeled substrate was [F18]FHBG (Alauddin MM, Conti PS. Nuclear Medicine & Biology. 25:175-80, 1998). In Panel A, the grp78 promoter is able to drive high level HSVtk expression in sizable solid tumors. A tumor was formed in the left (L) shoulder area in a BALB/C mouse by injecting s.c. 2 X 107 of G1NaGRP-HSVtk transfected TSA cells. The microPET scan was performed when the tumor was about 1.5cm in diameter. The red color denotes high HSVtk activity. In Panel B, the grp78 promoter is inducible by hypoxia activated by photodynamic treatment (PDT). Two tumors were formed simultaneously on the left (L) and right (R) shoulder areas of a BALB/C mouse the same as described in A. The microPET scan was performed when tumor sizes reached about 0.6 cm in diameter and about 12 hours after the tumor on the right had received PDT which induces hypoxia *in vivo*. The two tumors were of approximately the same size before PDT treatment and the relatively larger contour seen on the image on the R tumor is due to hemorrhage and edema after PDT treatment. The microPET analyses were performed in collaboration with the USC Medical Imaging Center and the UCLA microPET facility.

FIGURE 7

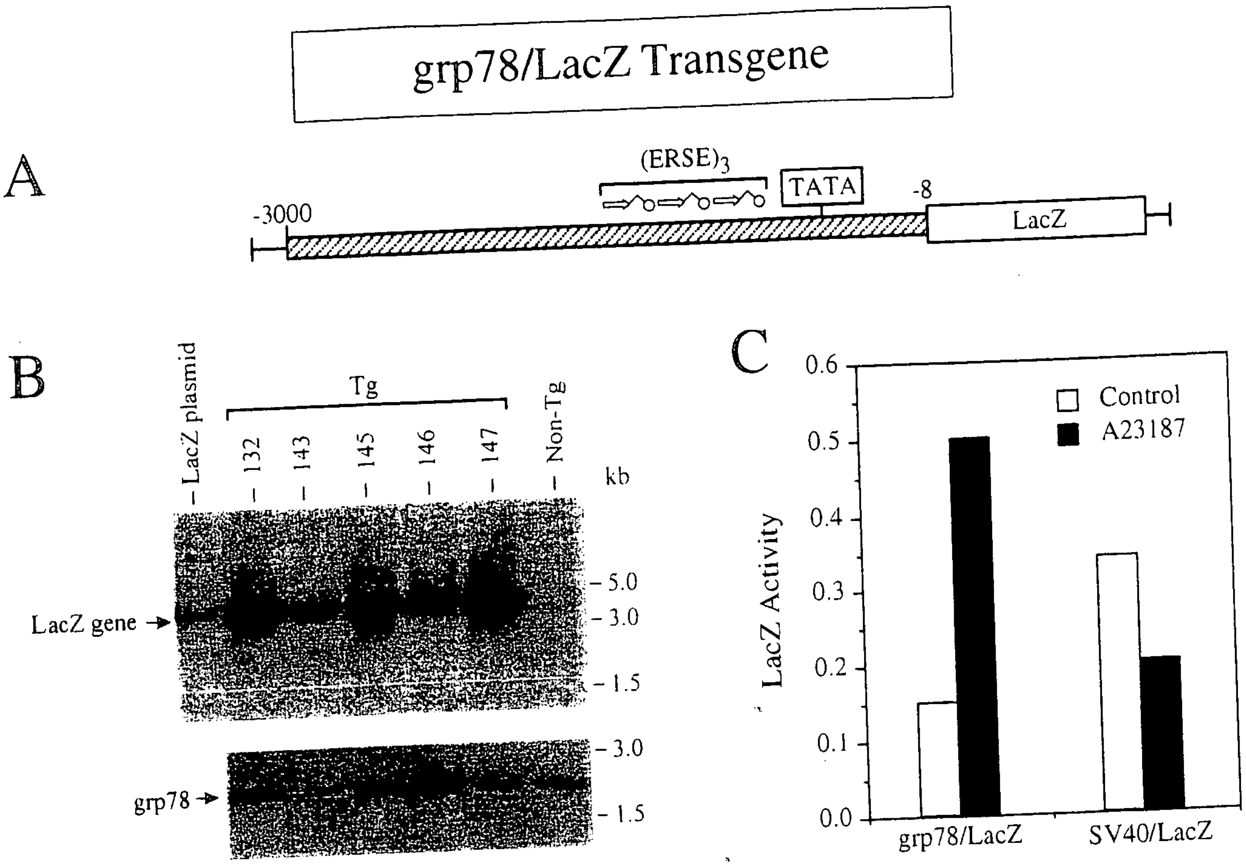

FIGURE 8

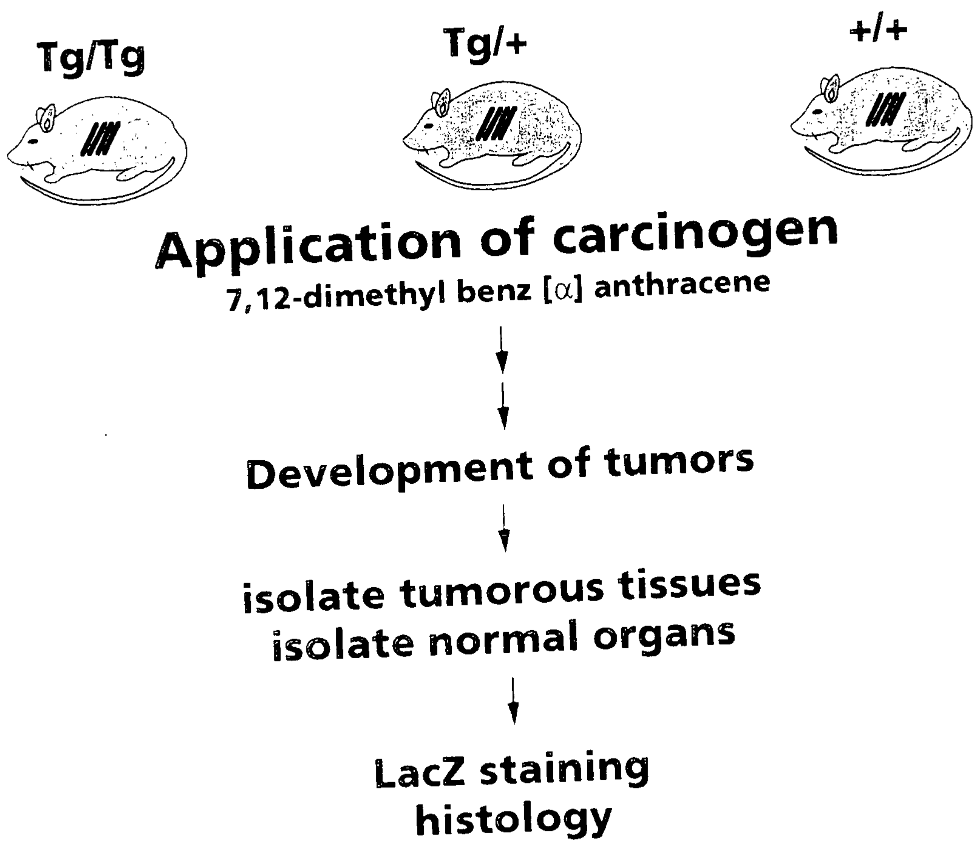

FIGURE 9

grp78/LacZ Transgenic Mouse

Normal Organs

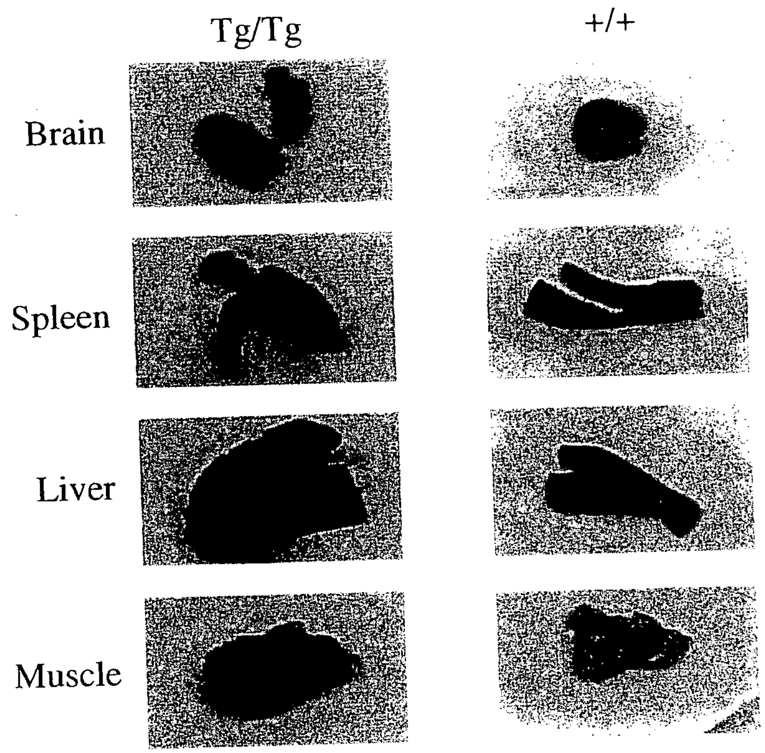

FIGURE 10

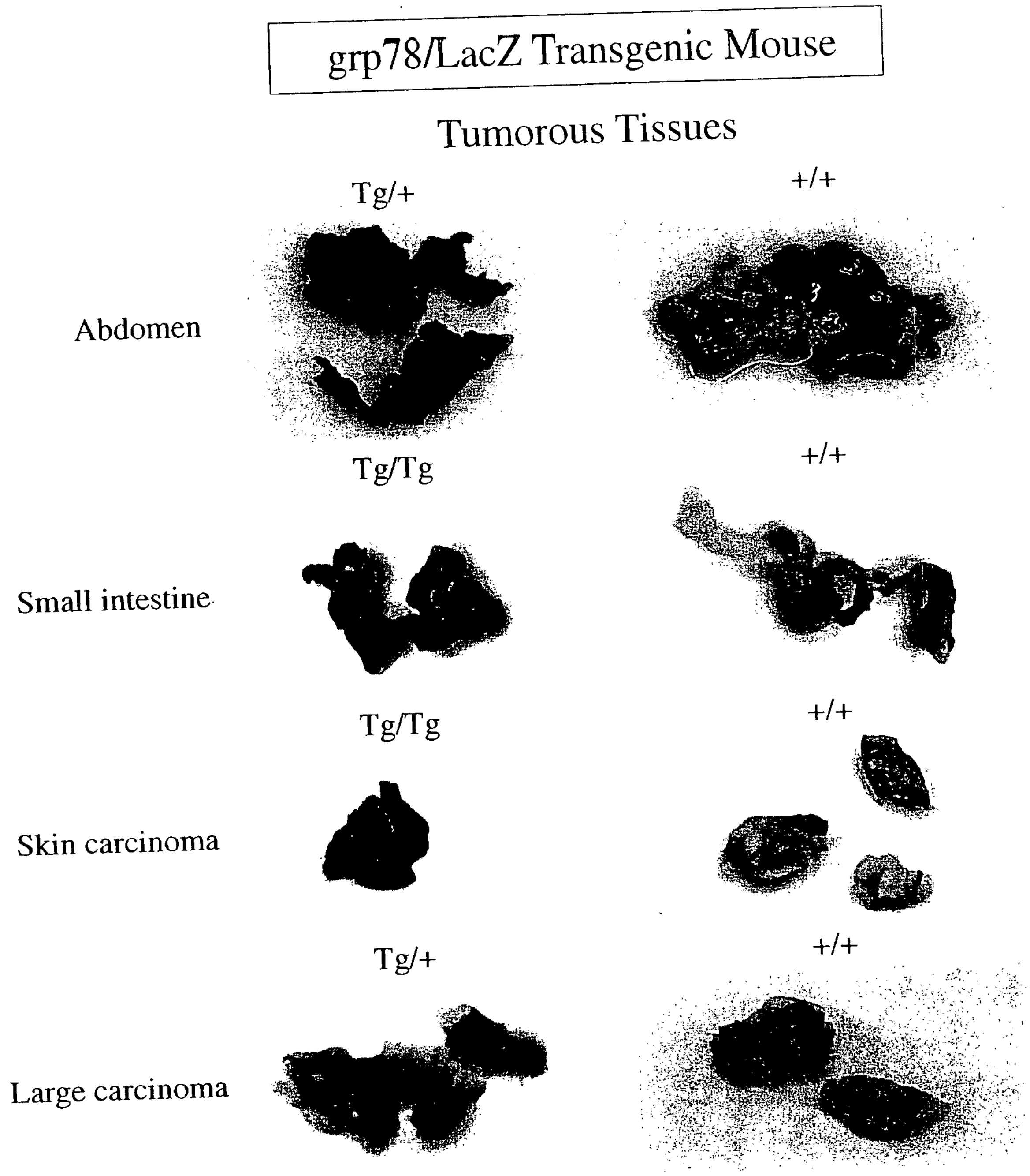
grp78/LacZ Transgenic Mouse
Tumorous Tissues
Tg/+
+/+
Abdomen
Tg/Tg
+/+
Small intestine
Tg/Tg
+/+
Skin carcinoma
Tg/+
+/+
Large carcinoma

FIGURE 11

grp78/LacZ Transgenic Mouse

Tumorous Tissues